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BRIEF COMMUNICATION

The effects of NaCl on growth, water relations, osmolytes and ion content in *Kochia prostrata*

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Abstract

The effects of NaCl (0, 50, 100, 150 and 200 mM) on growth, water relations, glycinebetaine, free proline, ion contents, stomata number and size of *Kochia prostrata* (L.) Schard were determined. Shoot and root fresh and dry matter, root and shoot length, relative growth rate, net assimilation rate, relative water content, water use efficiency, soluble sugars and glycinebetaine contents were not changed at low NaCl concentrations, but they were significantly decreased at 200 mM NaCl. The K^+, Mg^{2+} and Ca^{2+} contents, water potential, chlorophyll *a+b* and carotenoides contents, and stomata number and size were reduced already at low concentrations of NaCl. In contrast, the Na^+, Cl^- and proline contents increased several times with increasing NaCl concentration. *Kochia prostrata* is a salt tolerant species, the optimal growth of this plant occurred up to 150 mM NaCl. The mechanisms of salt tolerance in the plant may be balance among ion accumulation and production of glycinebetaine, proline, soluble sugars for maintenance of pressure potential.

Additional key words: glycinebetaine, halophyte, proline, stomata number and size, salt stress, salt tolerance, water use efficiency.

Many arid and semi-arid regions in the world contain soils and water resources that are too saline for most of the common economic crops. The utilization of halophytic plants pasture and fodder production in saline soils is the only economic solution presently available (Yeo and Flowers 1980).

Salt stress causes inhibition of growth and development, reduction in photosynthesis, respiration, and protein synthesis and disturbs nucleic acid metabolism (Levine *et al*. 1990). Decrease in uptake of K^+, Mg^{2+}, Ca^{2+} and thereby decrease in growth at higher Na^+ concentration have also been reported (Poonia and Virmani 1972).

In halophytes adaptation to salinity is associated with metabolic adjustments that lead to the accumulation of several organic solutes, such as sugars, polyols, betaines and free proline (Flowers *et al*. 1977, Gorham *et al*. 1981, Briens and Larher 1982). Accumulation of proline has been widely advocated for use as parameter of selection for salt stress tolerance (Storey and Wyn-Jones 1979). The proline shall be also used as energy and nitrogen storing suppressed (Sudhakar *et al*. 1993). The content of soluble sugars may be a good criteria for choosing of resistance species to salinity and drought, furthermore, monosacharides obviously have a major role in the initial response to salinity and drought (Kerepesi 1998). It has been reported that in response to salinity stress, glycinebetaine is accumulated in the cytoplasm of barley and sugar beet (Stewart and Lee 1974, Wyn-Jones and Storey 1981).

Kochia prostrata is a major source of protein and carotene for the feeding of domestic herbivorous animals in arid and semi arid regions. Regarding this point, less information is available about salinity resistance of *Kochia prostrata* at different growth stages. To realize the response of this species to salinity, the following experiment was conducted to find mechanisms of salt resistance of the plants.

Seeds of *Kochia prostrata* (L.) Schrad were collected during autumn 2002 from Esfahan Province in Iran. Plants were grown in 15 cm diameter pots in a growth chamber at 25/16 ºC (day/night), and a 16-h photoperiod

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Abbreviations: NAR - net assimilation rate, RGR - relative growth rate; RWC - relative water content, WUE - water use efficiency.
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(irradiance of 300 µmol m^{-2} s^{-1}. The plants were grown in half-strength Hoagland and Arnon solution (Moore 1960) until they reached about 10 cm in height. NaCl concentration was gradually increased by 50 mM NaCl increments at 2 d intervals to reach the maximum salinity of 200 mM NaCl after 10 d. The plants were grown in four replicates (20 plants per pot) in sand with 0, 50, 100, 150 and 200 mM NaCl. Fresh and dry mass of plant shoots and roots were measured at 30 d intervals after the highest salt concentration was reached. Dry mass was determined after drying for 48 h in a forced-draft oven at 70 ºC. Relative growth rate (RGR), relative leaf growth rate (RLGR), net assimilation rate (NAR), leaf area ratio (LAR), and leaf area/mass (LMR) were calculated according to Beadle (1993).

Water potential was by measured with a pressure bomb (*Model 1900*, *Sky Instruments*, Hertforshire, UK) on four randomly chosen shoots from each treatment (Richardson 1980). Leaf relative water content (RWC) estimation was done by incubating leaf samples (0.5 g) in 100 cm^3 of distilled water for 6 h and calculated according to Weatherley (1950). Stomata size and stomata number per unit leaf area were measured by microscope.

Chlorophyll *a*, *b* and carotenoid contents were estimated by Arnon method (1949) in leaf samples (0.25 g) homogenized in 5 cm^3 of aceton (80 %). Absorbance was recorded at 645, 633 and 470 nm (*Spectrophotometer CECIL Model 2000*, Cambridge, UK).

Free proline content in the leaves was determined following the method of Bates *et al*. (1973). Total soluble sugar was estimated by anthrone reagent (Yemm and Willis 1954). The K^+, Na^+, Cl^-, Mg^{2+} and Ca^{2+} contents were determined by *ICP* spectrometer (*Model 2001, GBC Ltd.*, Melbourne, Australia). Before measurement, 1 g of plant tissue was dried in an oven at 500 °C for 2 h. 10 cm^3 of distilled water and 10 cm^3 HNO_3 (1 M) were added to the ash. The volume of supernatant was increased to 50 cm^3 and filtered. Glycinebetaine estimation was done in according to Greive (1983). The absorbance was measured at 365 nm with UV-visible spectrophotometer. Reference standards of GB (50 - 200 µg cm^{-3}) were prepared in 2 M sulfuric acid.

The experimental design was completely randomized design with 4 replications of 5 treatments. The data was subjected to an analysis of variance and the statistical significance of the results was analyzed by the Duncan test.

Statistical analysis showed significant impacts of NaCl on *Kochia prostrata*, shoot length and root, fresh and dry matter, RGR, RLGR, NAR, LAR and LMR. NaCl concentration up to 150 mM did not alter these parameters, but they declined at 200 mM NaCl. The exception was root length which increased at 200 mM NaCl (Table 1).

Water use efficiency (WUE was significantly decreased at 200 mM NaCl, but it was constant at

Table 1. The effects of NaCl (0, 50, 100, 150 and 200 mM) on the growth and physiological parameters in roots and shoots of *Kochia prostrata*. Means ± SE, n = 4, means in a row followed by a different letter are significantly different ($P < 0.05$) according to Duncan test.

Parameters	0	50	100	150	200
Shoot fresh matter [mg $plant^{-1}$]	3500 ± 108a	3200 ± 115a	3100 ± 125a	3000 ± 232a	1400 ± 327b
Root fresh matter [mg $plant^{-1}$]	510 ± 31a	530 ± 40a	500 ± 35a	520 ± 21a	110 ± 12b
Shoot dry matter [mg $plant^{-1}$]	580 ± 34a	540 ± 24a	520 ± 15a	512 ± 38a	280 ± 13b
Root dry matter [mg $plant^{-1}$]	140 ± 15a	120 ± 12a	110 ± 16a	112 ± 12a	45 ± 7b
Shoot length [cm]	21.7 ± 2.6a	21 ± 2.4a	19 ± 2.5a	18.7 ± 2.2a	10 ± 2.4b
Root length [cm]	12.5 ± 1.5a	14 ± 2.4a	14.5 ± 2.2a	15 ± 1.8a	24 ± 2.8b
RGR [g g^{-1} d^{-1}]	0.29± 0.06a	0.27± 0.08a	0.27± 0.07a	0.28± 0.06a	0.11± 0.04b
RLGR [m^2 m^{-2} d^{-1}]	0.32± 0.02a	0.33± 0.02a	0.31± 0.03a	0.28± 0.02a	0.14± 0.03b
NAR [mg cm^2 d^{-1}]	0.49± 0.01a	0.51± 0.01a	0.49± 0.01a	0.48± 0.09a	0.22± 0.01b
LAR [m^2 kg^{-1}]	56.5 ± 1.4a	56.7 ± 1.7a	54.5 ± 1.2a	52.5 ± 1.1a	36.5 ± 1.2b
SLA [m^2 kg^{-1}]	64.2 ± 1.3a	63.5 ± 1.6a	64.5 ± 1.2a	62.2 ± 1.1a	28.5 ± 1.5b
WUE [mg(d.m.) $g^{-1}(H_2O)$]	54 ± 2.08a	50 ± 1.58a	50 ± 1.75a	48 ± 1.12a	18 ± 1.56b
Water potential [-MPa]	1.1 ± 0.08a	1.7 ± 0.11b	2.0 ± 0.11c	2.2 ± 0.12c	2.6 ± 0.14d
RWC [%]	92 ± 2.4a	91 ± 2.3a	89 ± 2.4a	88 ± 2.6a	50 ± 2.3b
Soluble sugars [mg g^{-1}(d.m.)]	27.5 ± 2.0a	39.5 ± 3ab	44.7 ± 2.9ab	54.2 ± 3.4b	146.7 ± 7.6c
Proline [µg g^{-1}(d.m.)]	150 ± 7.0a	195 ± 9b	252 ± 9c	299 ± 11d	396 ± 20e
Glycinebetaine [µg g^{-1}(d.m.)]	408 ± 25a	507 ± 26a	528 ± 38a	539 ± 64a	1015 ± 73b
Stomata number	10 ± 0.6a	11 ± 0.4a	13 ± 0.6a	14 ± 0.8ab	15 ± 0.6b
Stomata size [µm]	9.5 ± 0.6a	6.2 ± 0.6b	5.1 ± 0.4bc	4.9 ± 0.5bc	3.6 ± 0.3c
Chlorophyll *a* [mg g^{-1}(d.m.)]	0.77± 0.07a	0.55± 0.05b	0.43± 0.05bc	0.33± 0.04c	0.16± 0.03d
Chlorophyll *b* [mg g^{-1}(d.m.)]	0.73± 0.02a	0.49± 0.02b	0.42± 0.02c	0.19± 0.02d	0.09± 0.02e
Carotenoids [mg g^{-1}(d.m.)]	0.69± 0.02a	0.47± 0.03b	0.31± 0.02c	0.19± 0.02d	0.19± 0.03e